THE YIELDING CAPACITY OF COLCHICINE-INDUCED POLYPLOID SUGAR BEETS

Kenneth W. Hill

Department of Plant Science

University of Alberta

UNIVERSITY OF ALBERTA

FACULTY OF AGRICULTURE

The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "The yielding capacity of colchicine-induced polyploid sugar beets", submitted by Kenneth Wilford Hill, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

Professor [illegible]

Professor [illegible]

Professor J. R. Fryer

U N I V E R S I T Y O F A L B E R T A

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A THESIS
submitted to the University of Alberta
in partial fulfilment of the requirements for
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INTRODUCTION

During the last decade colchicine has been used extensively to induce polyploidy in plants. Stable polyploids of many plant genera have been produced and frequently these have been subjected to yield tests in comparison with their normal parental species.

Studies in the field of induced polyploidy seem to have had two main purposes: 1. to augment the general knowledge of cytogenetics and the mechanisms of heredity and evolution; and 2. to improve the productivity or utility of economic plants by doubling their chromosome number. The current study belongs in this latter division.

Polyploid sugar beets, resulting from colchicine treatments, have been produced in several countries (1, 13, 20, 25, 27). In Canada, Peto (20) induced polyploid strains of sugar beets and much of the material tested in the present work originated from this source.

At the time the present work was undertaken there were very limited published results on the yield performance of tetraploid sugar beets. The available results (20) (21)

were secured under conditions widely different from those which obtain in southern Alberta where climatic environment seems to be especially favorable to the synthesis of sucrose. Under Alberta conditions there has not been a depression in sugar content with increased size of roots, from improved cultural treatments, as has been the experience in other areas devoted to sugar beet production. All available evidence suggested that polyploid sugar beets could be expected to display an increase in root size and, consequently, a similar increase in the total production of sucrose.

LITERATURE REVIEW

The literature dealing with colchicine-induced polyploidy is already voluminous. As early as 1940 Dermen (10) reviewed 87 papers. Therefore it has seemed discreet in the present work to confine the review mainly to papers dealing either with polyploidy in sugar beets or with the effect of polyploidy on the chemical composition of crop plants.

Wellensieck (29) and Dermen (10) have made studies on the chronology of colchicine-induced polyploidy and have reported that the modern history of the utilization of this drug to induce polyploidy began in the laboratory of Professor A.P. Dustin, Brussels, Belgium. In 1934, F.J. Lits, (17) a student of Professor Dustin, studied the cellular reactions of animal tissue to treatment with colchicine. This was followed in the same laboratory by the work of Havas (15) in which he investigated the effects of colchicine on germinating wheat seedlings.

According to Dermen (10) the first successful colchicine induction of polyploidy in plants on this continent was reported by Blakeslee (5) and Blakeslee and Avery (6). Induced polyploidy has brought about widely variable changes in plants with respect to their physiology, chemical composition, morphology, and reproductive powers. Practically

all investigations of higher plants reveal that polyploids exhibit larger individual cells and fewer of them than normal diploids. Generally it has been found that the artificial induction of autopolyploidy is accompanied by a reduction in fertility.

Emsweller and Ruttle (11) have reported on induced polyploidy in floriculture which was the field first given attention by Blakeslee and Avery (6) and by Nebel and Ruttle (19). These authors state that induced polyploidy may have a place in the culture of ornamental plants since the new polyploid inductions have displayed several apparent advantages viz., size of flower, lateness in blooming (thus allowing a longer season), and quality of stem. The reduced fertility factor is obviously of less importance in those species which are propagated vegetatively.

Ross and Boyes (26) induced tetraploidy in flax and found that, while the size of seed was significantly increased, fertility was reduced to the extent that yields were definitely inferior. Evans and Johnson (12) report that induced tetraploids in sweet clover (<u>Melilotus alba Desr</u>.) displayed larger seeds and more vigorous seedlings although the apparent early increased growth rate was not maintained and there were no differences in yield when comparisons were made with normal diploid plants. Neither were there any differences in the nitrogen or coumarin

content. The incidence of larger than normal seeds arising from induced polyploids is an observation recorded by many investigations. (3, 4, 12, 21, 22). It seems highly probable that the increased vigor of polyploid seedlings would be accounted for in large measure by the fact that they arose from larger seeds.

With reference to the chemical content of induced polyploids, Sullivan (28) found that, compared to normal diploids, induced tetraploids of perennial ryegrass (*Lolium perenne L.*) contained less dry matter but significantly more sucrose and more total sugars. In white clover (*Trifolium repens L.*) octoploid plants were lower in both fibre and carotene than tetraploid plants. Increases of more than 40% in vitamin A activity of tetraploid corn meal as compared to meal made from original diploids has been reported by Randolph and Hand (22). Japanese investigators are quoted (23) as having increased the nicotine content of tobacco plants (*Nicotiana tabacum L.*) by 18% to 33% as a result of having doubled the chromosome number in common strains. Simultaneously, significant increases were noted in total nitrogen, fats, calcium, potassium, and magnesium, together with compensating decreases in carbohydrates, sulphur, and phosphorus.

Sugar beets were one of the earliest crops in which improvement was attempted by colchicine-induced polyploidy. In Sweden, Rasmusson and Levan (25) produced tetraploid sugar beets by immersing the very young shooting flower stalks in a test tube containing a 1% aqueous solution of colchicine for periods of 24 to 48 hours. The German workers Frandsen (13) and Schwanitz (27) treated the tops of full grown beets before bolting ensued. By this method they procured some tetraploid shoots among a large number of normal diploid shoots on each plant.

On this continent Artschwager (3) (4) treated sugar beet seed with an aqueous solution of 0.5% colchicine and subsequently selected approximately 100 individuals believed to be tetraploid out of several thousand seedlings obtained from treated seed. The seed yields from these tetraploid individuals were generally moderate or small and some plants did not set seed. The seed balls were predominantly larger than those from comparable diploids. Although there was great variation between the various individuals, nevertheless on the whole the tetraploids displayed much larger stomatal cells and pollen grains than the corresponding diploid material.

Abegg (1) (2) also produced tetraploid sugar beets and has reported on two results of yield tests comparing the tetraploid strains with their parental diploids.

The tetraploids were produced by soaking seed in a 0.4% solution of colchicine for 2 days and subsequently selecting the abnormal seedlings. First generation tetraploid offspring were obtained by crossing individuals which on the basis of pollen size were adjudged to be tetraploid. Of incidental interest is the fact that tetrasomic inheritance of the R - r hypocotyl color-factor pair was used as substantiation of tetraploidy among these offspring. As a result of yield tests in 1940, which included nine tetraploid strains and three parental diploids, in a randomized-block experiment, the author reports that the lowest yielding 4n strain averaged 2.51 pounds per beet contrasted to 3.07 pounds per beet for the comparable diploid parent; the highest yielding 4n strain produced beets of average weight of 3.14 pounds each compared to 2.81 pounds each for the corresponding parental diploid.

In single row plantings of tetraploids and parental diploids which were harvested and compared in pairs the tetraploids showed an average weight of 2.56 pounds and the diploids an average weight of 2.70 pounds. However, one tetraploid strain exceeded its parental diploid in root weight by 13.8%. This increase was statistically significant. The percentage of sucrose of the tetraploids was significantly lower than that of the diploids in the majority of the comparisons.

The 1941 results were quite closely corroborative of the 1940 data, and therefore led this investigator to conclude that doubling the chromosome number in sugar beets has not generally resulted in increased productivity over the normal diploid varieties. However the exceptional vigor of some 4n strains suggested to him the possibility of progress by discriminating selection.

Lynes and Harris (18) attempted several methods of inducing polyploidy in sugar beets and found that x-rays, calcium phosphate, and ethyl mercury phosphate capsules gave negative results. Colchicine was effective in both seed soaking and agar capsule treatments; soaking seed in ethyl mercury phosphate solution gave positive results as did irrigation treatments with sulphapyridine and sulfanilamide solutions.

In 1938 Peto and Boyes (20) treated young flowering branches of sugar beets with agar capsules containing 1% or 5% colchicine and thereby induced tetraploid sectors. Apparently these were subsequently pollinated by diploid pollen as a quantity of triploid seed was obtained and positively identified by chromosome counts of the seedling root tips. Sufficient triploid and comparable diploid plants were produced in the greenhouse to transplant into a small replicated field test. The yield results from this test were studied with great interest since they constituted the first field comparison of induced-polyploid beets with their parental diploids. The triploids exceeded the diploids

by 12.2% in root weight, 14.9% in indicated yield of sucrose, 17.8% in dry top weight, and were exactly equal in sucrose percentage. These differences were all statistically significant. The data were also studied in regard to the regression relationships between percentage of sucrose and root size. The slopes of the regression lines for triploids and diploids were significantly different. Triploids showed an average decrease of .17% of sucrose for each increase of 100 grams in root weight, with the diploids the comparable figure was .34%. The correlation coefficients for these two factors were -0.74 and -0.54 for the triploids and diploids respectively; both were highly significant.

Regression lines were also computed for the yield of sucrose per beet on weight of beet. These lines suggested a decided advantage for the triploids over the diploids. Whereas the diploid regression line began to fall into a decided curve from its mid-point indicating, that for large beets the yield of sucrose was not comparably high, the triploid relationship remained practically linear throughout indicating that it should be possible to produce very large triploid beets without any serious reduction in percentage of sucrose.

Subsequent to the investigations of Peto and Boyes (20) additional work with material from the same source has been reported on by Peto and Hill (21). In a randomized-block experiment including two parental diploids, a triploid,

hybrid triploid, tetraploid, and hybrid tetraploid, the polyploids displayed much more uniformity than their parental diploids. The hybrid tetraploid was significantly higher, in tons of roots per acre, than all other strains except one of its parental diploids. Generally speaking the polyploids were lower in percentage of sucrose than the diploids although this was ascribed in part to the degree of severity of defoliation by Cercospora leaf-spot. In spite of this the hybrid tetraploid ranked second in the production of sucrose per acre.

MATERIALS AND METHODS

The polyploid strains used in this study were produced by colchicine treatment of normal sugar beet seeds. The method consisted of placing a small quantity of seed on blotting paper in a petri dish; the blotting paper was then moistened with sufficient 0.4% colchicine to provide optimum germinating conditions and the seeds were allowed to germinate from 48 to 96 hours. Following this treatment all seeds were planted in soil contained in greenhouse flats. It was usually found that the germination percentage had been reduced about 50%. As the seedlings emerged they were rigorously selected and all normal appearing ones were discarded. The abnormal, but vigorous, seedlings were pricked out and planted in small pots and subsequently transplanted to the field. As the roots approached the size at which they are usually harvested an elimination was made on the basis of stomatal size. It was found that the area indices of stomata on tetraploid leaves were generally about twice as great as those on comparable diploid leaves, and plants with small stomatal cells were therefore destroyed.

At harvest time the surviving tetraploid roots and comparable diploid roots were removed to the greenhouse and so arranged by isolation and alternation that seed of

either triploid, hybrid triploid, tetraploid, or hybrid tetraploid would be produced. (In this paper, "hybrid triploid" means that a diploid of one variety and a tetraploid of another were crossed; in a triploid the parents were of different chromosomal constitution but of the same variety). At time of pollination individuals were again checked for ploidy using pollen size as the criterion.

In the spring of 1942, a field experiment was designed to test 17 different strains; these included five of triploid constitution, seven of tetraploid constitution, four parental diploids, and one unrelated diploid in common use in Southern Alberta. All strains except one were of European origin. The exception (Great Western) was developed by the Great Western Sugar Co., Denver, Colo. A list of strains with their chromosome constitution is presented below in Table I.

The experiment was located on land leased by Canadian Sugar Factories Ltd. near their processing factory at Picture Butte, Alberta.

TABLE I

Summary means of root weight, percentage of sucrose, and indicated sucrose per beet, of fifteen strains of sugar beets included in a polyploid yield test in 1942.

Strain	Acces. No.	Ploidy	Root Wt. oz.	% Sucrose	Indic. sucrose per beet Oz.
Buszczynski	H4005	3n	40.3	18.7	7.49
Buszcznyski	14113	2n	30.4	19.3	5.92
Buszcznyski	T4121	4n	37.0	19.1	7.09
Sandomiersko	13802	2n	33.9	19.6	6.59
Sandomiersko	T4116	4n	32.6	19.1	6.24
San. X Bus.	XH4128	3n	31.1	19.6	6.11
San. X Bus.	XH4129	3n	33.3	19.3	6.46
San. X Bus.	XT4126F1	4n	28.6	19.8	5.64
San. X Bus.	XT4107F_2	4n	45.3	19.2	8.66
Schreiber	13803	2n	32.7	19.3	6.32
Schreiber	T4109	4n	43.0	19.1	8.18
Great Western	14114	2n	38.6	19.8	7.64
Great Western	T4114	4n	39.3	18.0	7.03
G.W. X Schr.	XT4123	4n	47.7	18.6	8.86
Dobrovice	34102	2n	38.9	19.2	7.42

The field plan of the experiment was a complete randomized-block design with 10 replications of the 17 strains. A plot consisted of 10 hills 22 inches apart in 22-inch rows. Seeds were planted by hand on May 14 at the rate of 3 or 4 seeds per hill. The beets were later thinned to one plant per hill and subsequently cared for in the acceptable manner for commercial sugar beets in the area. They were irrigated twice. Two hybrid triploids from reciprocal crosses (Schreiber 4n X Dobrovice 2n) failed to germinate satisfactorily and therefore were discarded. At harvest time all beets from each plot were dug, cleaned, topped, and weighed and a pulp sample from each beet was composited to provide a sample for determination of the percentage of sucrose. The sucrose analyses were made in the laboratory of Canadian Sugar Factories Ltd. using the Sachs-Le Docte hot-water digestion method (9). Statistical reduction of the data follows the method outlined by Goulden (14).

EXPERIMENTAL RESULTS

The experimental results are shown in Table I under the following headings, average weight per beet, percentage of sucrose, and indicated sucrose per beet.

It will be noted from Table I that generally the polyploids produced larger beets than the diploids but the percentage of sucrose of the diploids was usually superior.

Analysis of Root Yield

The analysis of variance of the yield of roots of the various strains is presented in Table II.

TABLE II

Analysis of variance of individual root yields of polyploid and diploid sugar beets grown in 1942.

Variance due to	D.F.	Mean squares	F value
Replications	9	81.53	1.57
Strains	14	324.10	6.25 **
Error	126	51.88	
Total	149		

** - Significant beyond the 1% point.

Minimum significant differences: 5% - 6.4 oz.
1% - 8.4 oz.

In regard to yield of roots, the differences between varieties and within the various polyploid derivatives of some varieties were highly significant. In the Buszczynski strains the difference between the triploid and the diploid was highly significant, the tetraploid and the triploid differed very little, and the difference between the tetraploid and the diploid was significant. Similarly, the Schreiber diploid was highly significantly inferior to the tetraploid produced from it. The Sandomiersko and Great Western tetraploids displayed similar performances to their parental diploids.

The average root yields of the hybrid tetraploids and the hybrid triploids displayed an interesting variation. The hybrid triploids (Sandomiersko 2n x Buszczynski 4n, and its reciprocal) produced similar yields very closely approximating the average of the two parental diploids. This indicates no advantage for hybrid triploidy and further suggests that a tetraploid or a diploid is equally effective as a maternal parent. The Sandomiersko x Buszczynski hybrid tetraploid (XT 4107) produced very large roots averaging 45.3 ounces each in weight. This yield was highly significantly better than both the diploids and both the tetraploids of the parental varieties. This suggests considerable worth for hybridity coupled with tetraploidy. The strain under discussion (XT 4107) had been reproduced once from the original cross and was therefore an F_2 strain. As an F_1 this strain was included in the yield test at Vancouver in

1941 reported on by Peto and Hill (21) and displayed the same superiority. Its performance in the present test as an F_2 is evidence for the stability of this synthetic tetraploid.

The other Sandomiersko x Buszcznski hybrid tetraploid (XT 4126) was an F_1, but not the parental material of (XT 4107). These two strains were related only in that they were developed from hybridization of tetraploids produced at different times from the same diploid parental varieties. The very poor performance of XT 4126 compared to the superior performance of XT 4107 is not easy to explain. It does appear however that the potentiality of a polyploid is dependent upon the inherent capacity of the particular diploid individual from which it was developed. It is well known that all varieties of sugar beets are extremely heterozygous in genic material and therefore if, in the production of a polyploid, superior diploid individuals are selected fortuitously it is reasonable to expect that the polyploids would exhibit relative excellence. However in dealing with heterozygous material, it is obvious that there are also chances of selecting mediocre or poor material. This matter will be discussed subsequently at greater length.

The hybrid tetraploid Great Western X Schreiber (XT 4123) gave the highest yield of roots of any of the 15 strains included in this test. This yield was significantly higher than both the Great Western 2n and 4n and also the Schreiber 2n. There was no significant difference between

the Schreiber 4n and the hybrid 4n (XT 4123). The striking superiority of this hybrid tetraploid prompted its inclusion in a standard commercial variety test in 1943 and its performance in that test will be dealt with later.

Analysis of Percentage of Sucrose

All roots from each plot were sampled by removing a radial sector extending from the outside of each beet to its centre and running the full length of the beet. This was done with a power-driven rotary rasp. The samples from the ten beets were composited and analysed as outlined above. The analysis of variance of the percentages of sucrose is presented in Table III.

TABLE III

Analysis of variance of percentages of sucrose of polyploid and diploid sugar beets grown in 1942.

Variance due to	D.F.	Mean squares	F Value
Replications	9	1.87	1.83
Strains	14	2.29	2.24 **
Error	126	1.02	
Total	149		

** - Significant beyond the 1% point.

Minimum significant differences: 5% - .90%
1% - 1.17%

The percentages of sucrose of the various strains did not show as much variation as did the root weights. The 4n, 3n, and 2n derivatives of the variety Buszcznski showed no significant variability. The same situation existed between Sandomiersko 2n and 4n and also between Schreiber 2n and 4n.

The Great Western tetraploid was significantly inferior to its diploid parent in percentage of sucrose; the Great Western X Schreiber hybrid tetraploid displayed similar inferiority to the Great Western diploid. There were no significant differences in the performances of the Great Western X Schreiber hybrid tetraploid, the Schreiber diploid parent, the Schreiber tetraploid, and the Great Western tetraploid.

The hybrid tetraploids, Sandomiersko X Buszcznyski (F_1-XT 4126 and F_2-XT 4107) did not differ significantly from their parental diploids or from the tetraploids derived from the parental diploids. Neither was there an apparent significant advantage for the hybrid triploids of Buszcznski X Sandomiersko.

In general it may be said that increasing the chromosome number decreased the sucrose percentage of sugar beets. This conclusion agrees in direction with that of Abegg (1) who found some polyploid strains significantly lower than their diploid parents in respect to percentage of sucrose.

Analysis of Sucrose per Beet

The merchantable product from sugar beets is, of course, sugar or, more technically, sucrose. Therefore it follows that the total production of sucrose is the ultimate criterion by which a variety or strain must be judged. This value is the product of the two factors which have been discussed latterly, viz., root yield and percentage of sucrose. Frequently this value is termed "indicated available sugar per acre" and is obtained by computing the product of root yield per acre and percentage of sucrose. In the present study it has been decided to express this value as "indicated sucrose per beet".

The analysis of variance of sucrose per beet for which the means are given in Table I follows in Table IV.

It should be pointed out that the indicated available sucrose per beet is not all readily extractable by ordinary refining methods. Routine laboratory analysis also gives the percentage of solids in solution which are readily available as refined sugar. This value usually varies from 85% to 88% under Alberta conditions and is referred to as the "purity" of the solution. It is admitted that purities of the various strains may vary, but rarely has this variability exhibited significant proportions under Alberta conditions. Hence it has not been introduced into these calculations.

TABLE IV

Analysis of variance of indicated sucrose per beet of polyploid and diploid sugar beets grown in 1942.

Variance due to	D.F.	Mean squares	F Value
Replications	9	1.75	.88
Strains	14	9.80	4.92 **
Error	126	1.99	
Total	149		

** - Significant beyond the 1% point.

Minimum significant differences: 5% - 1.26 oz.
1% - 1.64 oz.

The analysis of variance of sucrose per beet indicated that in the strains derived from Buszczynski, the triploid significantly exceeded the diploid but there was no significant difference between the apparent sucrose per beet of the tetraploid compared to the parental diploid; neither was there a difference when the triploid and tetraploid were compared.

Sandomiersko 2n and its derivative 4n showed comparable sucrose production. The same was true of the Great Western 2n and 4n. However the Schreiber tetraploid yielded more sucrose per beet than the diploid to the extent that the difference was highly significant.

The hybrid tetraploid Great Western X Schreiber led all of the strains in the production of sucrose per beet. This strain was significantly superior to both its parental diploids and also to the Great Western tetraploid; in addition significant superiority over the Schreiber tetraploid was approached.

The hybrid tetraploid Sandomiersko X Buszczynski (ST 4107-F_2) having placed second highest in both root weight and percentage of sucrose also displayed the second highest production of sucrose per beet and in so doing significantly outyielded both of its parental diploids and their tetraploid derivatives. This suggests an added advantage for tetraploidy combined with heterosis. This consideration will be referred to in more detail later. Unluckily enough, the F_1 hybrid tetraploid of similar origin (Sandomiersko X Buszczynski XT 4126) performed very poorly and thus detracts seriously from the faith which the performance of the F_2 would instil in the hopeful breeder.

The hybrid triploids showed no significant difference when compared to their diploid parental material.

The standard 2n normal variety, Dobrovice Select (34102), which was included somewhat as a measuring stick showed approximately an average performance in comparison to all the other strains in the test. Its values for root weight, percentage of sucrose, and indicated sucrose per beet were very close to the appropriate general means of the experiment in all three analyses.

Performance of Two Superior Tetraploids in Standard Variety Tests

The results of the polyploid yield test just reviewed suggest the superiority of two hybrid tetraploids viz., Sandomiersko X Buszczynski (XT 4107) and Great Western X Schreiber (XT 4123). These strains were therefore selected for further study. The hybrid tetraploid (XT 4107), by virtue of its performance in the yield test reported by Peto and Hill (23), was included in the regular commercial variety test of Canadian Sugar Factories Ltd. in 1942. The other superior hybrid tetraploid (XT 4123) was included in the regular variety test of the same company in 1943. In both of these years the commercial variety test consisted of a complete randomized block experiment with six replications. There were six normal diploid varieties and one hybrid tetraploid each year. The yield results in tons of sugar per acre (means of 6 replicates) are shown below in Table V.

Thus it will be seen that the hybrid tetraploid, Sandomiersko X Buszczynski (XT 4107) stood at the bottom of all other varieties in the 1942 commercial variety test while the other hybrid tetraploid, Great Western X Schreiber (XT 4123), led all commercial varieties in the 1943 test.

TABLE V

Comparison of tons of sugar per acre produced by one hybrid tetraploid and six normal diploid varieties in a commercial variety experiment in each of the years 1942 and 1943.

Variety	Tons of sugar per acre 1942	1943
G.W. X Schreiber 4n (XT 4123)		3.28
S.K.E.	2.60	3.21
Kuhn	2.56	3.25
R.G.O.T.	2.46	3.03
Alta 2	2.45	
Alta 3		3.15
Pioneer	2.21	2.90
Dobrovice	2.35	2.86
Imperial		2.30
Sand. X Busc. 4n (XT 4107)	1.99	

It will be recalled that these two hybrid tetraploids XT 4107 and XT 4123, performed very similarly (Sucrose production per beet - 8.86 oz. and 8.66 oz. respectively) in the polyploid yield test of 1942 which has been referred to in detail.

There was one deliberate source of variation between the polyploid yield test of 1942 and the commercial variety tests of 1942 and 1943. In the polyploid test the beets were grown in 22" rows and the spacing between beets within the row was also 22", this was done to eliminate competition; in

the commercial variety tests the rows were spaced 22" apart but the beets within the rows were at 12" spacing as in ordinary commercial production. No doubt this difference in spacing had some effect on the behavior of these two hybrid tetraploids but in order to explain their relative performance on this basis alone it must be assumed that the Sandomiersko X Buszczynski (XT 4107) responded very well to wide spacing but very poorly to close spacing and that the Great Western X Schreiber (XT 4123) responded very well on both wide and close spacing. Since it is not felt that differences in climate or soil type between the years 1942 and 1943 could account for much variation, no explanation other than spacing is offered for this wide discrepancy in performance.

It should also be said to the credit of the hybrid tetraploid (XT 4123) that its diploid parents Great Western and Schreiber were tested in earlier commercial variety trials and discarded because of their inferiority to some of the varieties that occurred in the test in 1943 and which were exceeded by the Great Western X Schreiber 4n hybrid.

<u>Polyploidy versus Heterosis</u>

Throughout this discussion there has frequently arisen doubt as to whether the significant differences were due to polyploidy or heterosis or a combination of

the two. It is not possible to definitely assign the causal effects of indicated superiority at the present state of research and in view of the limited data available. However all relevant data have been summarized and are presented below in Table VI. This table includes a summary of results of indicated sucrose per beet from 4 separate experiments viz., polyploid yield tests in 1942, 1943, and 1944 and a yield test of diploids and their inter-varietal hybrids in 1945. The normal diploid variety Schreiber occurred in all tests and its yield of sucrose per beet has been taken as 100 in each test and the yields of all other varieties in each test bear the same relation to 100 as their actual yield in each test bore to the appropriate yield of Schreiber.

TABLE VI

Comparative yield ratios of sucrose per beet of various diploid, hybrid diploid, polyploid, and hybrid polyploid strains of sugar beets grown in several different tests.

Strain	Acc. No.	Ploidy	Relative yield
Schreiber	13802	2n	100
	1945 hybrid yield test (8 reps)		
Schreiber X Kuhn		2n	83
Kuhn		2n	88.2
	1943 polyploid test (10 reps)		
Schreiber X Dobrovice	XH 4130	3n	117.5
Sandomiersko X Schreiber	XH 4246	3n	112.8
G.W. X Schreiber	XT 4123	4n	107.3
Schreiber	T 4109	4n	104.7
Sandomiersko X Schreiber	XH 4245	3n	80.6
	1942 polyploid test (10 reps)		
G.W. X Schreiber	XT 4123	4n	140.2
Schreiber	T 4109	4n	129.4
Great Western	14114	2n	120.9
Great Western	T 4144	4n	111.2
	1944 polyploid test (10 reps)		
Schreiber X Dobrovice	XH 4130	3n	123.7
Sandomiersko X Schreiber	XH 4246	3n	116.1
G.W. X Schreiber	XT 4123	4n	99.7
Schreiber	T 4109	4n	93.9

Admittedly this table has many gaps. One would like to have seen all of the varieties going to make up the polyploids occurring in the tests as diploids and also as inter-varietal diploid hybrids. Only in such a way could an appraisal be made of the relative worth of polyploidy and heterosis. From Table VI it has been calculated that all tetraploids showed an average index of 109.3 and all hybrid tetraploids had an average index of 115.7. No significance can be established for this difference but it may be indicative of a trend.

GENERAL DISCUSSION

The yield results of colchicine-induced polyploid sugar beets obtained from the reported experiment were comparatively variable and do not warrant extensive claims for superiority due to induced polyploidy. However some of the induced-polyploid strains were sufficiently promising to indicate that this method of breeding cannot be justifiably ignored.

The hybrid tetraploid (XT 4107) produced 42% more sucrose per beet than the average of its two parental diploids. This increase was due entirely to an increase in root size since the hybrid tetraploid had 41% larger roots than the parental average while in percentage of sucrose the hybrid was a little more than 1% lower. When similar comparisons were made for the other hybrid tetraploid (XT 4123) it was found that this strain showed 27% increase in sucrose per beet; 34% increase in root weight and a decrease of 5% in percentage of sucrose. These are somewhat more promising results than were secured by Abegg (2) who found three autotetraploid strains which exceeded their related diploid stocks in root weight by 15% but which due to a comparable decrease in percentage of sucrose showed no net gain in sugar production. The extensive photoperiod of Southern Alberta is apparently very favorable to the synthesis of sucrose (16). As a

general rule vigorous growing "tonnage"[*] varieties display a much higher sucrose percentage under Alberta conditions than under more southerly conditions. This may account in part for the better performance of tetraploids in Alberta as compared to Beltsville, Maryland (2).

Comparing triploids and diploids Peto and Boyes (20) reported that the two types were exactly equal in percentage of sucrose while the mean increase of triploids over diploids for root weight was 12.2% and for sucrose production 14.9%.

In only one case has a sugar beet investigator (2) reported a significant increase in percentage of sucrose attributable to polyploidy. However Sullivan (28) working with ryegrass (Lolium perenne L.) found a highly significant difference, in percentage of sucrose and also total sugars, for tetraploids over diploids.

Sugar beet breeders are in general agreement that it is relatively easier, by standard breeding methods, to increase the yield of roots in a variety than to increase the percentage of sucrose. Many investigators have suggested, as a possible explanation of this, that a great many more factors are concerned in the inheritance of sucrose percentage.

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* - Sugar beet varieties are divided according to performance into three general classes: tonnage type, sugar type, and normal (intermediate) type.

Randolph (24) has shown that in maize, chromosome doubling of inbred lines greatly reduced their vigor while tetraploids derived from normal cross-pollinated stocks are equally or more vigorous than the original stocks. This writer postulated that the lack of vigor in tetraploid strains derived from inbreds was due to the doubling up of deleterious genes which were already homozygous at several loci. Since normal or increased vigor accompanied tetraploids derived from normally heterozygous (cross-pollinated) stock he concluded that homozygosity per se contributed to lack of vigor while heterozygosity in itself may be responsible for much of the vigor or heterosis exhibited by hybrids.

Abegg (2) found a striking reduction in vigor when inbred strains of sugar beets were doubled in chromosome number. The tetraploids derived from inbreds yielded only one-half the weight of roots produced by the inbred diploid lines, whereas tetraploids produced from normal cross-pollinated strains were variable but generally quite similar to their parents in yield of roots. It seems therefore that the postulations of Randolph (24) would apply with equal force to sugar beets. Extending this line of reasoning to the experimental results of the present work it will be noted that the autotetraploids were similar in performance to their diploid parents, thus agreeing with Abegg (2), but two hybrid tetraploids were definitely superior particularly in root weight.

Since the present study has provided only very limited data on the performance of hybrid polyploid sugar beets and since this phase of polyploidy apparently has not been explored by other investigators, it is not possible to reach a definite conclusion. However, in accordance with Randolph's theory (24) it seems reasonable to hypothesize that the parental diploid materials used in this test contained a usual number of deleterious growth genes in varying degrees of heterozygosity. The performance of the autotetraploids may have been dependent on the number of deleterious genes which were doubled in their derivation whereas the superiority of the hybrid tetraploids could be explained by the assumption that they were heterozygous at most of the loci containing deleterious genes.

It is well known that sugar beet germ plasm is extremely heterozygous, extensive improvement in a variety frequently being made by mass selection (7). Even in a so-called "pure" variety the variation in size and percentage of sucrose of individual roots is comparatively large. Furthermore it has been shown (8) that normal sugar beet varieties vary greatly in their combining ability. It is to be expected also that individuals within any one variety would very likely show differences in combining ability. In light of these considerations and bearing in mind that the polyploid strains

studied in the present work were generally derived from very few seeds it becomes less difficult to understand the extreme variability exhibited in the performance of some of the strains. That polyploid strains superior to their parental diploids have been produced seems to have been amply proven; however, it seems to have been equally well proven that it would not be possible, given the same varieties of original material, to produce a similarly superior strain with any degree of regularity.

The present results indicate that it is not possible to predict the performance of an induced polyploid on the basis of the yielding capacity of its parental diploid variety. However when tetraploids were produced from inbred strains by Abegg (2) there seemed to be a positive correlation between the yield of the induced tetraploid and the yield of the inbred strain. This would be expected since the inbred line would likely be relatively homozygous and there would be less variation among the individuals within the strain. However in the present work, tetraploids produced by random selection of a few seeds from a standard variety seemed to bear no orderly relation in performance to that of the parental variety. A similar result was reported by Abegg (2). In the present state of knowledge it does not appear possible to explain the variable excellence of induced-polyploid strains except by attributing it to the fortuitous selection of a few

parental seeds which perhaps were not necessarily representative of the variety.

This inference suggests a more hopeful approach toward sugar beet improvement by induced polyploidy. Normal diploid varieties must be selected and improved and their combining ability determined to the extent that their breeding behavior can be predicted; the evolution of polyploids from this superior material should be far more fruitful of consistent improvement.

Those who would attempt an appraisal of the worth of induced-polyploid sugar beets would find it difficult, from available evidence, to establish their superiority. Their percentage of sucrose has been generally poor. Their overall performance has been generally inferior. But though it cannot definitely be said with truthfulness that polyploids are an improvement over normal strains, nevertheless, one cannot justifiably overlook the manifest expression of increased vigor in some polyploids; their frequent excellence in root size, the occasional accompaniment of increased sucrose percentage, and the combinations of these which have produced strains of decided promise in experimental material.

CONCLUSIONS AND SUMMARY

(1) Polyploids of sugar beets can be readily produced by treatment of the seed with colchicine and subsequent selection of the abnormal individuals on the basis of morphology, cell size, pollen size, and chromosome number.

(2) Colchicine-induced tetraploids are relatively stable and fertile and produce satisfactory quantities of viable seed.

(3) Generally speaking colchicine-induced polyploids studied in this experiment tended to exceed their parental diploids in root size.

(4) Percentage of sucrose was affected relatively less than root size by chromosome doubling and although there were relatively few significant differences these were never in favor of the polyploids.

(5) On the basis of sucrose per beet, one tetraploid and two hybrid tetraploids showed highly significant superiority over the appropriate parental diploid material.

(6) It was not possible to separate the effects due to polyploidy and heterosis and both are thought to be operative in the superior hybrid tetraploid strains.

(7) The performances of the polyploid strains were extremely variable and it is suggested that this can be explained in part by the extreme heterozygosity of the parental material as well as the paucity of individuals from which the polyploids were developed.

(8) Induced polyploidy may improve the productive capacity of sugar beets but results will likely be more consistent if only proven diploid material is used in the synthesis of the polyploids.

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